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Neurotrophins and cholinergic enzyme regulated by calpain-2: New insights into neuronal apoptosis induced by polybrominated diphenyl ether-153

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ABSTRACT

Polybrominated diphenyl ether-153 (BDE-153) has been demonstrated to induce neuronal apoptosis in rat cerebral cortex and primary neurons. Neurotrophins and cholinergic enzymes play critical roles in the neuronal survival, maintenance, synaptic plasticity and learning memory, however, their roles in neuronal apoptosis following the BDE-153 treatment remain unclear. In this study, we firstly explored the possible predominant pathway underlying the neuronal apoptotic induced by the BDE-153 treatment in rat cerebral cortex, by measuring expression levels (mRNA and protein) of p53, caspase-3, 8, 9, calpain-1, and calpain-2, detected the levels (protein contents and mRNA) of neurotrophins including brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4), and measured acetylcholinesterase (AchE) and choline acetyltransferase (ChaT) activities in rat cerebral cortex and primary neurons following BDE-153 treatment with or without pretreatment with inhibitors. Results showed that the neuronal apoptosis induced by BDE-153 was dependent on p53, and dependent on more calpain-2 than caspase-3 in the cerebral cortex of rats. Following the BDE-153 treatment, the protein contents and mRNA levels of BDNF, GDNF, NGF, NT-3, and NT-4, as well as the AchE and ChaT activities were significantly decreased in the cerebral cortex and primary neurons when compared to the untreated group. When pretreated primary neurons with calpain inhibitor PD150606 or cyclin-dependent kinase (cdk5, the downstream complex of calpain) inhibitor Roscovitine, the neurotrophins contents and activities of ChaT and AchE were reverted, along with the improvement of neuron survival compared with BDE-153 treatment alone. We conclude that neurotrophins and cholinergic enzymes were regulated by the calpain-2 activation and its downstream cdk5 pathway, and which was involved in the neuronal apoptosis induced by the BDE-153 treatment.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) have been widely used throughout the world in products such as electronics, cables, carpets, furniture, etc. Humans are being exposed to PBDEs via inhalation and ingestion (Allgood et al., 2017; Zhou et al., 2016), although PBDEs were phased out in the manufacture of new products. Prenatal or postnatal PBDE administration in experimental rats and mice resulted in neurotoxicity including learning and memory deficits, spontaneous activity impairment, and habituation disruption (Viberg et al., 2003; Viberg et al., 2006; Zhang et al., 2013), with neuronal apoptosis being one of the main mechanisms (Zhang et al., 2013). However, the underlying mechanism for neuronal apoptosis induced by PBDE remains largely unknown.

Neurotrophins is a family of small proteins involved in neuronal growth, maintenance, survival, and synaptic plasticity in brain, and

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plays a critical role in the pathogenesis of neurodegenerative and neuropsychiatric disorders (Castren and Kojima, 2017; Caviedes et al., 2017). The predominant neurotrophins in the study field of health and disease include brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4). BDNF is a key factor with multipotent impact on brain development, signaling transduction and synaptic plasticity under physiological and pathological conditions (Kowianski et al., 2018), has been used as a potential monitoring marker or therapeutic target for Alzheimer's disease or mood disorders (Bjorkholm and Monteggia, 2016; Castren and Kojima, 2017; Gerenu et al., 2017; Sarabi et al., 2017). Increased BDNF expression in hippocampus and cortex is associated with the cognition improvement in rats (Callaghan et al., 2017), and BDNF is protective for neuronal apoptosis in rats (Yuan et al., 2017). Cholinergic system is well-known to regulate cognition and memory function, and acetylcholinesterase (AchE) activity is reported to predict memory function and mediate memory deficits following white matter lesions in human (Richter et al., 2017). Cognition dysfunction in mice or rats is attenuated via decreasing AchE activity and increasing choline acetyltransferase (ChaT) activity in hippocampus (Haider et al., 2016; Xu et al., 2016). However, few studies have reported the possible roles of neurotrophins and cholinergic enzymes in the process of neuronal apoptosis induced by the BDE-153 treatment.

As known, there are several different neuronal apoptotic pathways, which are labeled by some representative proteins including p53, aspartate-specific cysteine proteases (caspases), and calcium protein activated neutral proteases (calpains), etc. Therefore, we usually called neuronal apoptosis as p53 or non-p53 dependent apoptosis, caspase-dependent or calpain-dependent. Generally, a certain kind of pathway is dominated and overlapped with other pathways. In this study, we aim to explore the possible predominant pathway underlying the neuronal apoptotic in the cerebral cortex of rat following the BDE-153 treatment, and then measured the changes of neurotrophins (at mRNA and protein levels) and cholinergic enzyme activities in rats' cerebral cortex and primary neurons, in order to find a new potential mechanism of neuronal apoptosis following the BDE-153 treatment.

2. Materials and methods

2.1. Chemicals and reagents

2, 2′, 4, 4′, 5, 5′-Hexabrominated diphenyl ether (BDE-153, purity 99.9% by GC/MS) was purchased from AccuStandard, Inc. (NewHaven, CT, USA). ELISA kits for caspase-3, 8, 9, brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4), acetylcholinesterase (AchE) and choline acetyltransferase (ChaT) were purchased from Shanghai Jianglai Biological Technology co., LTD, China. Rabbit polyclonal antibodies against p53 (D2H9O) (32532s), Calpain-1 Large Subunit (Mu-type) (2556s), Calpain-2 Large Subunit (M-type) (2539s) were from Cell Signaling Technology, USA. One Step SYBR PrimeScript RT-PCR Kits were purchased from Takara Biotechnology (Dalian) Co., LTD, China. Calpain inhibitor PD150606 (97%, HPLC) and Cdk5 inhibitor Roscovitine (98% TLC) were purchased from Sigma-Aldrich Co., China.

2.2. Animals and treatment

Ten nulliparous Sprague-Dawley rats (10 weeks of age) were obtained from the Experimental Animal Center of Shanxi Medical University (license number: 754) and individually housed in a plastic cage under standard conditions (room temperature 20–22 °C, humidity 40–70%, lights from 07:00 to 19:00) since the first day of pregnancy. Rats were allowed free access to food and tap water. The animal experimental protocol was approved by the Ethics Review Committee for Animal Research of Shanxi Medical University. The birthday was denoted as postnatal day 0 (PND 0). Following an established procedure in our laboratory (Zhang et al., 2017; Zhang et al., 2013), 60 male pups were randomly assigned into 4 groups (15 pups/group) according to their litters and body weights as follow: the control (olive oil vehicle) group, and the three BDE-153-treated groups at doses of 1, 5, and 10 mg/kg body weight. Pups were administrated intraperitoneally (i.p.) with BDE-153 solution or vehicle once at 0.1 ml/10 g body weight at PND 10. The suckling pups were housed with their mother until weaning at PND 21. The weaned pups were co-caged with their peers in the same group (6 pups/cage), with body weight, food and water consumption recorded daily until euthanasia at PND 70. All procedures were conducted in accordance with the Guide for Care and Use of Laboratory Animals from the National Research Council (US) Committee (2011).

In animal experiment, BDE-153 solution was prepared freshly by dissolving in olive oil, and sonicated for 30 min at room temperature before dosing. The dose range was set on the basis of our preliminary experiment and the referenced LOAEL (the lowest observed adverse effect level) 0.9 mg/kg in neonatal mice (Viberg et al., 2003).

2.3. Primary neuron culture and treatment

As described in our previous publication (Zhang et al., 2017), primary neurons were isolated from a newborn rat's brain, grown in the culture medium of Dulbecco's Modified Eagle Medium (DMEM) (containing 10% fetal bovine serum, 10% horse serum and 100 U/mL penicillin-streptomycin) in a culture plate or flask pre-coated with poly-L-lysine (0.1 mg/mL), and incubated in a humidified incubator with 5% CO_2 at 37 °C. Cytosine arabinoside (2.5 μM) was added to inhibit the glial cell proliferation and differentiation at 48 h. Well-grown and comparable 80–90% confluent neurons out of the same batch were classified and treated with medium (blank control), Dimethyl sulfoxide (DMSO, vehicle control), or BDE-153 at doses of 10, 20 and 40 μM. DMSO accounted for 0.3% (v/v) of the total incubation medium. In parallel, additional groups of cells were pretreated at 30 min prior to the BDE-153 treatment with calpain inhibitor PD150606 (97%, HPLC Sigma-Aldrich Co., China) or Cdk5 inhibitor Roscovitine (98% TLC, Sigma-Aldrich Co., China). After the BDE-153 treatment for 48 h, neurons were harvested for the detection of cell apoptosis, the neurotrophins mRNA and protein levels and cholinergic enzyme activities.

In vitro, BDE-153 was dissolved in DMSO (Sigma-aldrich), stocked at 4 °C in the dark, and diluted with serum-free culture medium up to the final concentrations (0, 10, 20, and 40 μM) before treatment. PD150606 or Roscovitine was dissolved with serum-free culture medium as well.

2.4. Sampling

At PND 70, the cerebral cortex was instantly separated following anesthesia with sodium pentobarbital. A 0.5 cm^3 prefrontal cortex was immediately digested into single cell suspension for cell apoptosis measurement using flow cytometry. The other cerebral cortex were cut into pieces and stored at −80 °C until used for measuring lactate dehydrogenase (LDH) activity, mRNA levels of p53, caspases-3, 8, 9 and neurotrophic factors, and determining the activities of caspases-3, 8, 9, and cholinergic enzymes, and protein levels of p53, calpain-1 and calpain-2.

The neurons were harvested after 48 h treatment for measuring neuronal apoptosis, neurotrophins contents, and cholinergic enzyme activities, and the supernatant were collected for measuring LDH leakage.

2.5. Cell apoptosis and LDH detection

Single cell suspension from prefrontal cortex tissue or cultured